Development of Ozone Technology Fish Storage Systems for Improving Quality Fish Production

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Abstract- Development of ozone technology fish storage system (OTFIS) to maintain the quality of the fish has been done. The ozone technology fish storage system (OTFIS) can be applied in the fishing boat, fish storage in the Fish Auction Market, fish storage in the means transport for fish. In this research, we produced a prototype that was integrated by the fish container and ozone generator. We used Dielectric Barrier Discharge Plasma (DBDP) technology for ozone generator. The characterization of ozone generator has been carried out and showed that ozone generator can produce ozone concentration from 0.5 ppm to 12 ppm. The ozone concentration in the area is very suitable to be used in food technology. The ozone treatment was performed on red tilapia representing the white-fleshed fish with ozone concentrations from 0.5 ppm to 4.5 ppm. The results of this treatment showed that organoleptic and TVBN of fish were still appropriate with Indonesian National Standard (INS). Beside that, TPC testing has been done on the red tilapia showed that the higher the ozone concentration the smaller the remaining of bacteria colonies. Similarly, for a variable of time, the longer the exposure to ozone the less the remaining of colonies. The results of this study have been adapted to realize of OTFIS with a size of 50 cm x 50 cm x 100 cm (250 liters) and can accommodate as much as 100 kg of fish.

Keywords: fish, storage, quality, plasma, ozone

I. INTRODUCTION

The Indonesian marine fisheries potential spread in nearly all parts of the existing Indonesian waters as the territorial sea of the archipelago and the waters of Exclusive Economic Zone (EEZ). The Indonesian sea area is estimated at 5.8 million km^2 with the longest coastline in the world at 81.000 km and the cluster of islands as much as 17.508. This geographical situation has the potential of fish estimated as many as 6.26 million tons per year. This amount can be detailed as much as 4.4 million tons can be caught in the waters of Indonesia and 1.86 million tons can be obtained from Indonesian Exclusive Economic Zone (IEEZ) waters.

According to the Ministry of Maritime and Fisheries, the Indonesian marine fisheries nominal value of 57.69 trillion IDR, or the equivalent of 6.48 percent of GDP in 2012, while the value of economic activity around 255.3 billion IDR. The potential to grow by 7 percent if compared to the second quarter 2013 to the second quarter of 2012 [1]. In addition to marine fisheries, Indonesia also has great potential for freshwater fisheries. The sector of freshwater fish aquaculture in Indonesia has the potential to be developed through the extension and intensification. The commodities of freshwater fish farming such as catfish, carp, tilapia, and freshwater ornamental fish have a fairly high demand in domestic and export markets. Export markets demand a positive impact on the increase in Indonesia's foreign exchange.

The fishery export is closely related to the quality of fish delivered, while the quality of the fish was associated to fish storage technology from the whole process of traveling fish caught up to the consumer. Currently, there are several methods for preserving fish using methods including drying, curing, salting, fermentation, and the most commonly used is cooling. Most people in ways that can be harmful to health, for example by using formalin for preservation of fish.

From the above discussion it can be concluded that, fish storage technology has not been able to keep the quality of the fish. The new technology is needed to resolve the issue. The alternative is ozone technology. The utilization of appropriate ozone technology and ozone dose right is believed to be used for storage technology in the whole his journey process begin caught to the consumer. The ozone technology has been developed for the water sterilization process, vegetable or food, medical equipment, and various other applications. In 1995, ozone was declared safe by Generalize Recognized as Safe (GRAS) or was generally recognized to be safe by the FDA (U.S. Food and Drug Administration) for the treatment of drinking water bottles. These applications are then developed as "GRAS" for food processing by some experts the last few years [2].

Ozone can be produced using two electrodes fed by high voltage with gas fill between the two electrodes in the form of pure oxygen gas or air. Between the two electrodes is covered by a dielectric material in order to avoid the occurrence of arc discharge. The factors that can affect ozone formation in general are voltage, dielectric materials, the pressure, the configuration system of the plasma reactor, and a gas in the plasma reactor [3,4,5]. Ozone formation involves mechanism of ionization and recombination both dissociation and association. The reaction of ozone formation can be occured in a plasma reactor [6].

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Bacteriocidal effect (substances that can kill bacteria) of ozone has been tested against a variety of organisms, including Gram-positive bacteria (*Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus, Enterococcus faecalis),* Gram-negative *(Pseudomonas aeruginosa* and *Yersinia enterocolitica give), yeast (Candida albicans give Zygosaccharomyces Bacilli), spore (Aspergillus niger)* and vegetative cells. Microbial inactivation is a complex process. Ozone can affect glycoproteins and or glycolipids membrane , the binding of membrane enzyme, and may be able to damage proteins and DNA. Microorganisms is attacked through the process of disruption (disorder) in the cell or disintegration that causes lysis process of the cells. Lysis process by ozone is a fast inactivation mechanism compared to other disinfectants that require disinfecting agent to permeate through the cell membrane [2,7,8].

According to Voidarou 2006 [2], microorganisms and mycotoxin decontamination process depends on the concentration of ozone given, exposure time, pH, and moisture content. Disinfecting properties of ozone has been tested by several researchers. For example, Tiwari et. al., 2009 [8], which examined the effectiveness of ozone against the grain pests such as *Tribolium castaneum* and *Ephestia elutella* and the *Coliform bacteria*, *Micrococcus*, *Bacillus* spores ,fungi, and so on. In his report [8], Tawari et. al. wrote that the respiratory system to be the entrance of ozone into the body of the insect. Ozone causes oxidative tissue damage (even at low concentrations), DNA damage, and changes in the pulmonary system, bronchial sensitivity, membrane oxidation or in vivo mutation. Increased respiratory system with increased temperature can result in excessive gas exchange due to an increase in metabolism and respiration rate.

In a different study [9], Ran et al., test the effectiveness of ozone against *Criptosporidium* (intestinal protozoa, one of the causes of diarrhea) and the Giardia parasite protozoan. In addition, Ran also investigated the mechanism of *Cryptosporidium* cell destruction by ozone using scanning electron microscopy (SEM) at time 0, 60, 120, and 480 seconds. As a result, the *Cryptosporidium* cell suffered the greatest damage in 480 seconds.

Research on microbial inactivation of bacteria and molds (fungi) using ozone has been done by several researchers. For example, Hardin et al. [10] who observed the effectiveness of gas ozone and liquid against the bacteria *E. coli*, *Coliform* bacteria, and fungi. Tests proved, disinfectant properties (the *E. coli* and *Coliform* bacteria) gas ozone is lower than the liquid ozone. Similarly, when testing of molds (fungi), which the total of reduced mold perfectly in 15 minutes using liquid ozone while in the gas ozone still leaving approximately 1 log CFU/gram. Type of mold identified degraded populations include *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus parasiticus* [9,11]. The effectiveness of ozone test against *E. coli* was also conducted by Patil et. al., in 2009 [12], where they used ozone to sterilize orange juice from bacterial contamination.

Ozone treatment on bacteria indicator in water has done by a Voidarou team [2], with samples taken from several points of irrigation in Epirus. Voidarou proved a decrease of resistance in the *E. coli* and *Enterococcus sp* after ozone treatment of 30 minutes,with ozone concentration in the range from 0.15 to 0.20 mg/lt . Voidarou also wrote some of the most important factors that influence the process of inactivation of *E. coli*, namely: ozone concentration, exposure time, and RH.

Utilization of ozone have been made to improve the safety and quality of salmon [13]. Quality of sardines can be maintained properly in a long time through a combination of ozone treatment and ice [14].

Ozone was reported to be effective in detoxifying and eliminating mold and mycotoxins such as aflatoxins, patulin, cyclopiazonic acid, D secalonic acid, and ochratoxin A. The factors that determine the ability of ozone to kill insects, rot causing bacteria in food, removal toxic mold, and decontamination depends among others, ozone concentration, exposure time, and pH [8.11,15]. Utilization of ozone and refrigeration for the storage of fish has been able to maintain the quality of fish for example was reported by [14, 16, 17, 18,19].

Ozone is able to inhibit the process of decay in fish. The process of fish preserving in principle is the inhibition of spoilage in fish. Immediately after the fish dies, there will be changes that lead to decay. The changes are primarily due to the activity of enzymes, chemicals, and bacteria. Enzymes contained in the fish body will remodel the body parts of fish and resulting changes in taste (flavor), smell (odor), form (appearance), and texture. Chemical activity is the meat fat oxidation by oxygen. The oxygen contained in the air oxidizing fatty fish meat and causes the rancid smell.

Changes caused by bacteria triggered by the occurrence of damage to the components in fish by enzyme and chemical activity. Chemical activity produces simpler components. This condition is preferred bacteria that trigger the growth of bacteria on the body of fish. In fact, the process of quality deterioration is very complex ongoing. The crochet hooks with each other, and work simultaneously. To prevent damage quickly, it must always be avoided occurrence of three activities simultaneously. Researches on ozone disinfectant for bacteria continue to grow.for example, Johansson and colleagues reported that ozone was also effectively used to degrade the cariogenic bacterial populations, such as *S. mutans*, *L. casei*, and *A. naeslundii* [20]. According to Pascual et. al., [21], the effectiveness of ozone to eliminate microorganisms not only depend on the amount given, but also the amount of residual ozone is still stored in the medium. Residual ozone is a ozone concentration that can still be detected in the medium after ozone exposure to target surface. In recent years, research on plasma technology and applications, including ozone produced by the plasma technology has been developed. Some research applications of plasma technology has been successfully carried out spin-off [22].

All of the research results have been reported, there are no integrates of the ozone capabilities as disinfectant against microorganisms and the ability of the fish shelf life in the fish container that can be used in fishing boats, fish auction market, and fish delivered in the means transport. This paper reports the results obtained from research on the development of ozone technology fish storage system (OTFISS) that integrated fish containe, ozone generator based on Dielectric Barrier Discharge Plasma (DBDP), blower and ozone circulation system. The quality of fish in the OTFISS has been compared with INS according to fresh fish.

II. METHOD

Figure 1 shows a series of experiments in this study. ozone has been generated by Dielectric Barrier Discharge Plasma produced with spiral-cylindrical (SC) configuration reactor. DBDPwas generated by AC high voltage voltages up to 25 kV and frequency up to 23 kHz. Electrical parameters of DBPP determined through a voltage divider (HV Probe DC max voltage DC 40 kV; AC 28 kV code number EC1010, En G1010). Electrical signal from the probe detected by oscilloscope GOS-653, 50 MHz. Electric current generated in the reactor was measured using a multimeter (Sunwa TRXn 360). Photo from experiments was taken by using a CCD camera (Creative, DV Cam 525d).

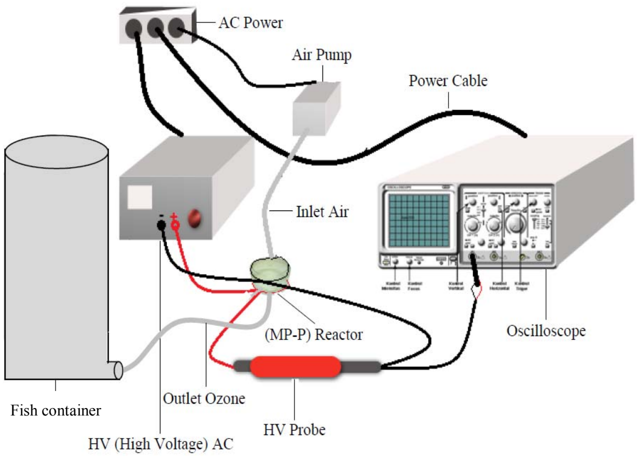

Figure 1. Experimental sut up series

The concentration of ozone generated by DBDP reactor with spiral-cylindrical configuration was measured using an ozone monitor (Quant Ozone "2"). Measurements were taken at applied voltage variations and multiple diameter of cylinder electrode/pyrex tube. Determination of Total Volatile Base Nitrogen (TVBN) and Trimethyl Amin Nitrogen (TMA-N) used standard SNI 2354.8.2009 issued by BNSI [23]. To test the freshness of fish by using SNI 01-2729.1.2006, organoleptic, fresh fish - Part 1st: Specification issued by BNSI [24].

III. RESULTS AND DISCUSSION

The primary objective of this research was the optimization of ozone reactor condition with DBDP technology to conduct advance studies to optimize the production of ozone. We varied some parametric studies such as the length of the reactor, the length and the number of windings of the spiral coils electrode, the length and thickness of the dielectric barrier. Finally by varying the voltage values, the ozone concentration was obtained that suitable for a fish container with a certain size. These studies are expected to get optimum ozone generator for OTFISS and it can mentain the quality of fish.

3.1. Characterization of Reactor

In this research, various types of high voltage used for production of ozone in the DBDP reaktor have been realized. We realized high voltage sources up to 40 kV and a frequency up to 25 kHz and AC high voltage, 25 kV with a frequency of 7 kHz. Both types of high voltage sources have been tested to produce ozone in the DBDP reactor.

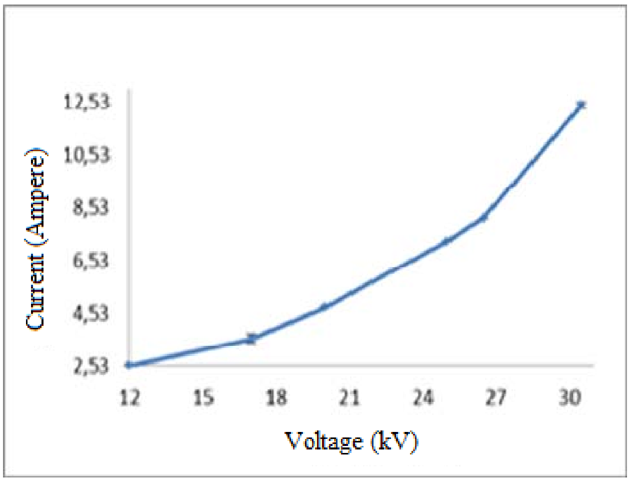

Figure 2. Electric current as function of applied voltage for DBDP reactor spiral-cylinder, for diameter of Pyrex tube of 3 cm

During all the experiments, Pyrex tube with a thickness of 1 mm was used as a dielectric barrier. The air gap was set to 1 mm and the frequency of the applied voltage is fixed at 23 kHz. Figure 2 show electric current as function of voltage for SC reactor with diameter of Pyrex tube were 3 cm and figure 3 for diameter of Pyrex tube were 5 cm.

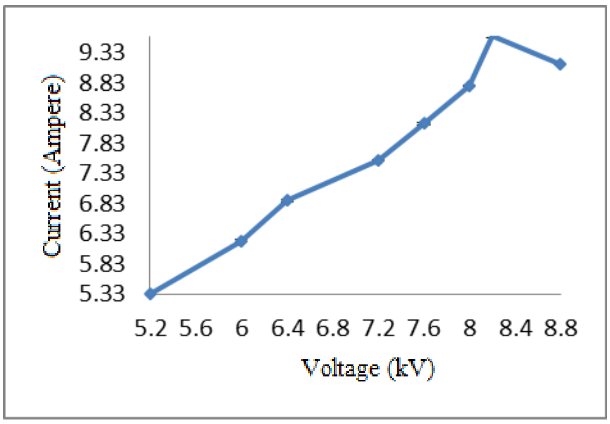

Figure 3. Electric current as function of applied voltage for DBDP reactor spiral-cylinder, for diameter of Pyrex tube of 5 cm, with 160 spiral coils anf length of reaktor 40 cm.

3.2. Characterization of Ozone Generator

This research was began with realize the ozone generator. The ozone generator uses a reactor with DBDP (Dielectric Barrier Discharge Plasma) technology. DBDP reactor that was made have three sizes Pyrex diameter: diameter of 2 cm with a length of 15 cm; diameter of 3 cm with a length of 15 cm; and diameter of 5 cm with a length of 40 cm. This reactor has been tested against the ozone concentration produced.

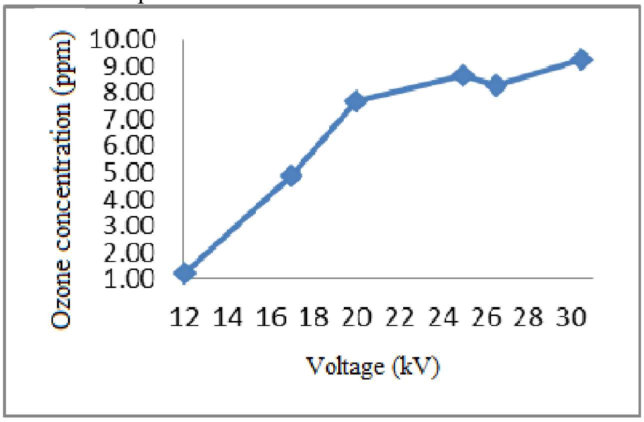

Figure 4. ozone concentration as function of applied voltage, for diameter of reactor of 3 cm with a length of 15 cm.

The graph showed in Figure 4 shows that the greater the concentration of ozone with increasing voltage. Figure 5 shows the concentration of ozone as a function of voltage for Pyrex diameter 5 cm, length 40 cm, and for a number of spiral coil electrode inside a Pyrex coil 160 convolution.

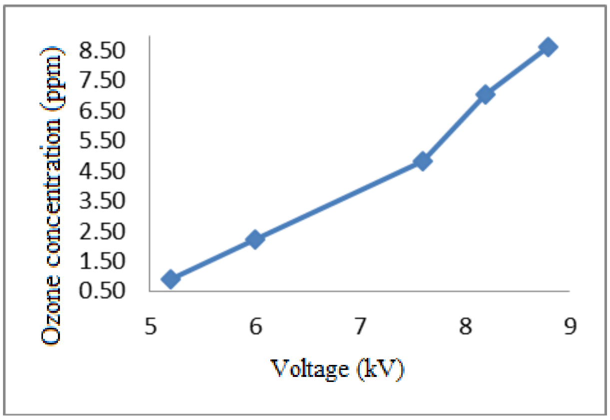

Figure 5. ozone concentration as function of applied voltage for diameter of pyrex D= 5cm, with lenght l= 40cm and spiral coils L=160.

3.3. Organoleptic Testing of Fresh Fish

Organoleptic testing on fresh fish, in this case red-tilapia, has been done. The tests carried out on samples that have been treated by ozone at different concentration and time varying. The results obtained show that the treated fish was better than fish control, and the treated fish is still in the range of fresh fish category based on SNI 01-2729.1-2006 published by BSNI [23].

The curve in Figure 6 shows comparison of fresh fish, called "control", and the fish that have been treated by ozone. For example, in 30 minutes of treatment given to each applied of ozone concentration: 0.5 ppm, 1.0 ppm, and 1.5 ppm, and always there is a "control". Organoleptic values for control fish at any time variation were about 9, while the fish that have been treated by ozone have organoleptic value were about 8 on all of time variation. According to SNI 01-2729.1-2006, about the specification of fresh fish [23], this value is still in the range of fresh fish category.

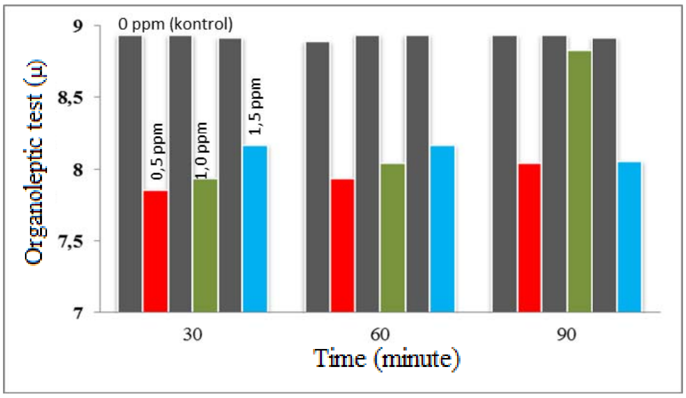

Figure 6. the results of organoleptic test for red tilapia with variation of ozone concentration and time of treatment by ozone. The red, green, and blue curves show ozone concentration for respectively time of treatment: 30 minutes, 60 minutes, and 90 minutes.

3.4. Total Volatile Base Nitrogen (TVB-N) test

The test of TVBN has been done against to treated samples with variation of ozone concentration: 0.5 ppm, 1.0 ppm, and 1.5 pm. The uses of ozone for this entire experiment show the improvement to value TVBN of fish.

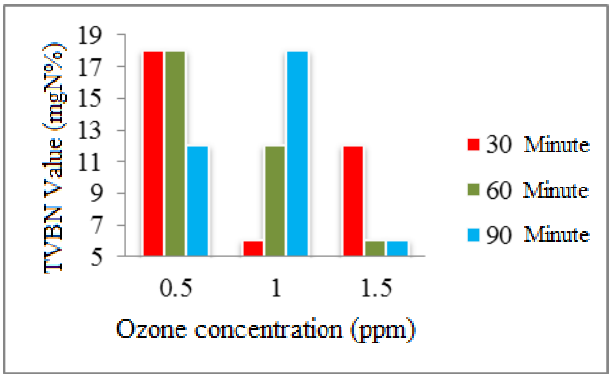

Figure 7. The test result of TVBN for red tilapia with variation of ozone concentration and variation of time of treatment by ozone until 90 minutes.

The curve in fig.7 shows TVBN values for each treatment with ozone concentration of 0.5 ppm, 1.0 ppm, and 1.5 ppm, with time variation of 30 minutes, 60 minutes, and 90 minutes. In this result, we found that the TVBN values was in the range of 6 to 19. According to SNI 2354.8:2009 [22], standart for fishery products, these values is in the range of fresh fish category.

3.5. Test of TPC

Microbial growth, in treated fish, is very slow, the reduction of growth is highly significant compared to "control". The number of micro-organism colonies was very small compared to fish that not being treat by ozone. The decrease was reaching to 2 logCFU /g (from the order of 104 CFU/gram became the order of 102 CFU/gram).

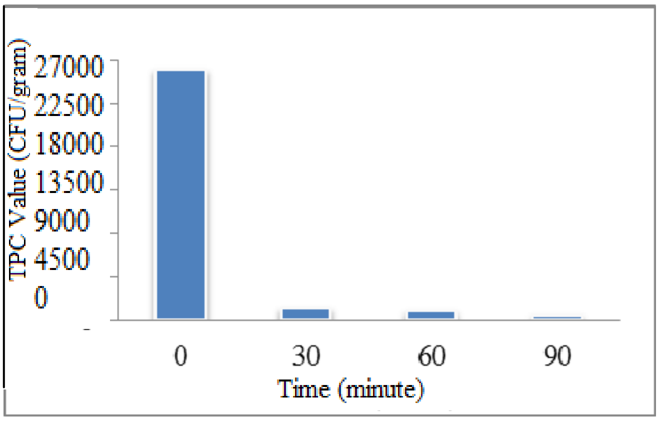

Figure 8. The result of test of TPC for treated red tilapia, with dissolve ozone concentration of 2,5 ppm.

Figure 8 and figure 9 shows that the timing of treatment by ozone of 30 minutes had decrease 2 logCFU/gram of bacteria, and more decrease in 60 minutes and 90 minutes. According to research by Crowe [12], treatment by ozone for 1,5 ppm (mg/liter) does not does not destroy the fat that contents in fish. This study using ozone concentration of 2.5 ppm - 4.5 ppm, and it is necessary to research about the fat content.

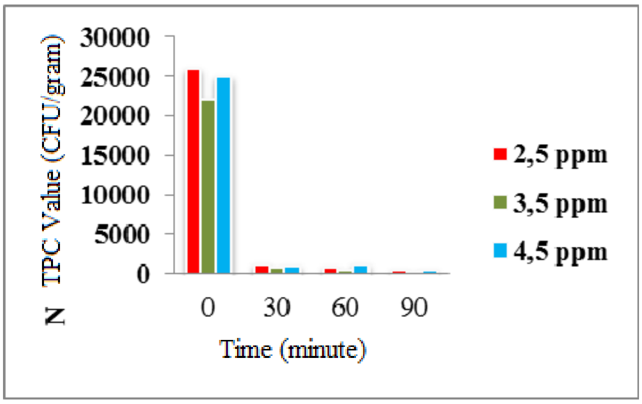

Figure 9. The result of test of TPC for treated red Tilapia, with time variation of dissolve ozone concentration of 2,5 ppm, 3,5ppm, 4,5ppm.Figure 9: Test results on the TPC red tilapia with ozonation time variation of dissolved ozone concentration of 2.5 ppm, 3.5 ppm and 4.5 ppm

3.6. Realization of OTFIS

The results of this research have shown that ozonation in fish can maintain the quality of fish. The results of this study are used to realize the ozone technology fish storage system (OTFIS) for fish trading centers. OTFIS size that successfully realized is 50 cm x 50 cm in long base and lid size, and height of 100 cm (see figure 10). Realized OTFIS made of stainless steels standard for food.

Figure 10. Two OTFISS with size 50 cm x 50 cm x 100 cm.

OTFISS equipped with wheels for easy removal at the site utilization. The design has been adapted for placement ozone generator, blowers, and high voltage. OTFISS used slurry ice (mixture of ice 40 % and water 60 %) and ozone dissolved into slurry ice inside og OFTISS. OTFISS also made watertight not leak and the placement of the generator room. OTFISS direct field testing by comparing the fish directly stored in the ozone generator was turned and comparing with OTFISs, that ozone genarator did not turn on. The results of OTFISS test shows in table below.

Table . The results of field tests for OTFISS

Parameter tested	Treatment to fish in OTFISS	
	Treated by ozone Every 6 hours, with 90 minutes treatment	Control
	Slurry ice (40 %ice + 60 % water), the composition was being renewed every 6 hours	Slurry ice (40 % ice + 60 % water) the composition was being renewed every 6 hours
	No external isolation	No external isolation
	3 days in treatment	3 days in treatment
Value of organoleptic	$7{,}55 \le \mu \le 8{,}23$	$6{,}27 \le \mu \le 6{,}73$

IV. CONCLUSION

Ozone generator with Dielectric Barrier Discharge Plasma technology which has been realized can generate ozone (0.5 ppm-12 ppm) appropriate with the concentrations region needed for ozone technology fish storage system (OTFIS). The ozone technology fish storage system (OTFIS), which is associated with cold storage system using ice slurry (a mixture of ice and water with a composition of 40 % ice and 60 % water).

Tests on samples of red-tilapia ozonized, we found that organoleptic of sample was still meet the fresh fish standard according to SNI. Ozonation on red tilapia can maintain the value of TVBN and reduce the growth of microorganisms in fish. The growth of bacterial colonies in the fish can be controlled by exposure of ozone. The tendency of the longer time of ozone exposure and higher the concentration of ozone gave less the remaining colonies of bacteria.

Organoleptic and TVBN of fish ozonized is still in the range of fresh fish category and still appropriate with Indonesian National Standard (INS) based on SNI 01-2729.1-2006 published by BSNI. The results of the study have been adapted for use in the realization OTFIS, with a realizing OTFIS size of 50 cm x 50 cm x 100 cm, and give product that OTFIS still able to maintain the freshness of the fish after 3 days stored.

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